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# Evaluating Foliar Application of Calcium Nitrate, Calcium Chloride and Boric Acid on Physiological Disorders of 'Kaleh - Ghoochi' Pistachio

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A R T I C L E I N F O

*Keywords:*

Blankness;
Deformed nuts;
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A B S T R A C T

Pistachio suffers from some physiological disorders such as fruit abscission, endocarp lesion, blankness, non-split, early-split and deformed nuts. In this experiment, the effects of calcium nitrate (2 gr/lit), calcium chloride (2 gr/lit), boric acid (200 ppm) and their combination treatments were investigated on physiological disorders of 'Kaleh-Ghoochi' pistachio. Foliar application was applied one week earlier and two weeks later than full bloom. Then, physiological disorders and enzyme activities, such as catalase (CAT), poly phenol oxidase (PPO), peroxidase (POD), phenylalanine ammonilyase (PAL) and total phenol level of nut-shells and proline level of leaves were tested. Results specified that all applied treatments diminished fruit abscission. Also, all treatments, excepting boric acid, diminished endocarp lesion and non-split nut disorders but there were no considerable differences between the treatments. Results specified that all applied treatments decreased the blank nut but there were no considerable differences between the treatments. In this research, the lowest deformed nuts with 10.38 percent and early split nut with 2.48 percent were observed by application of both calcium nitrate and boric acid. Meanwhile, the highest split nuts with 56.55 percent were obtained by application of calcium nitrate and boric acid. Generally, all treatments especially use of both calcium nitrate and boric acid diminish the catalase, poly phenol oxidase, peroxidase, and phenylalanine ammonilyase enzymes activities and moreover total phenol of nut-shells and proline of leaves.

## Introduction

Pistachio (*Pistacia vera* L.) has some physiological disorders such as fruit abscission, endocarp lesion nut, production of the blank nut, non-split, early-split and deformed nuts (Ferguson *et al*., 2005; Sajadian and Hokmabadi, 2011). Fruit abscission is one of the critical physiology disorders in pistachio. This occurrence causes economic damage in pistachio orchards. Sometimes the amount of fruit drop in pistachios is over 80 percent. Usually pistachio trees produce plenteous of flowers and pollen grains, but most of flowers and fruits are fallen during beginning fruit development and only small amount of the fruits are persist on pistachio clusters. Researchers recognize that physiological status and last year nutritional status, kind of medium stresses, lacking pollen grains, rainfall during pollination, weakness of pollination or fertilization, disruption of embryogenesis, contention for carbohydrates sources and inadequate plant growth substances stimulus the

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fruit abscission in pistachio trees (Stephenson, 1981; Acar and Eti, 2007).

Other studies specified that application of free polyamines and organic acids diminish the percentage of fruit abscission (Khezri *et al*., 2010; Kamiab *et al*., 2015). Endocarp lesion disorder was announced from pistachio orchards in newly years. These disorders cause economic destruction to pistachio producers. Researchers reported that calcium scarcity causes endocarp lesion in pistachio nuts (Sajadian and Hokmabadi, 2011). Hence limited research has been done about the endocarp lesion disorder in pistachio nut. Researchers believed that generation of blank nuts in pistachio is dependent to embryo miscarriage during the growing season, different medium stresses and parthenocarpic occurrence (Crane and Nelson, 1971; Polito and Pinney, 1999). Some reports specifies that generation of blank nuts has been depended on multiple factors such as unsuitable pollination, unsuccessful fertilization, spring rain-fall in pollination stage, inadequate nutrition, also kind of stresses during the kernel enlargement (Crane and Iwakiri, 1981; Ferguson *et al*., 2005).

In addition, application of L-arginine have been shown to remain ameliorative effect on fruit abscission and physiological disorders of pistachio nut (Eslami *et al.*, 2019).

Khezri *et al*. (2010) believed that exogenous application of polyamine such as spermidine decreased the percentage of blank nuts. Shell spiriting in pistachio nut is associated to multiple factors such as orchards management type of cultivars, optimal irrigation and nutrition, crop load, medium situations, harvest time and level of plant growth adjustments (Crane and Iwakiri, 1981; Polito and Pinney, 1999; Ferguson *et al*., 2005; Khezri *et al*., 2010). In non-split nut, kernels are advanced but the shell does not cleave and this is not desirable for consumers. Some of the researchers reported that endocarp cracking in pistachio nuts is associated to kind of pistachio cultivars and pollen parents, crop load, harvest time, optimal irrigation and nutrition, kernel advance and free polyamines (Polito and Pinney, 1999; Ferguson *et al*., 2005; Khezri *et al*., 2010). Early-split nuts have split peels and shells in the pistachio nuts. It happens in the orchard before harvest time and kernels are exposed to biotic factors such as fungi especially aflatoxin and this is not desirable for consumers. Other studies specified that wrong management, medium stresses, imbalance and inadequate irrigation and nutrition can interfere in boosting of early-split nuts (Doster *et al*., 2001; Hosseinifard and Panahi, 2006).

Deformed nuts are tiny and poorly shaped nuts that diminish the pistachio market.it has been reported that mechanical damage, medium stresses, disease and insect assaults can interfere in boosting of deformed nuts (Niven *et al*., 1994; Fabbri *et al*., 1998). Reduce production in pistachio orchards are dependent to orchards management and numerous physiological disorders that related to different biotic and abiotic stresses (Ferguson *et al*., 2005). To deal with environmental stresses, plants are used from enzymatic and non-enzymatic process (Pazuki *et al*., 2015). Catalase, poly phenol oxidase, peroxidase, and phenylalanine ammonilyase are antioxidant enzymes which are produced versus stress situations (Queiroz *et al*., 2008; Amna *et al*., 2010; Kim and Hwang, 2014; Siddika *et al*, 2015). Phenolic complexes and proline are non-enzymatic mechanisms which are considered important in the interplay of plants with surroundings. Since most pistachio orchards in Iran are commonly constructed in arid areas with high salinity of soil and water. So ameliorating conditions of soil, water and nutrition quality can diminish the disorders in pistachio orchards.

From horticultural view point, it has been made clear that exogenous application of essential elements such as boron (B) and calcium (Ca) can interfere in boosting of quality and the yield of some fruit crops (Pradubsuk, 2011; Madani, 2014). Moreover, Enhancing effect of foliar application of potassium and zink on walnut and pistachio crop yield have been well demonstrated (Keshavarz *et al.*, 2011; Norozi *et al.*, 2019). Calcium as a necessary nutrient has main role in plants especially cell construction and

operations (Barker and Pilbeam, 2007). Some researchers indicated that calcium could boost anthracnose resistance in dragon fruit (Ghani *et al*, 2011). Researchers reported that using of calcium could diminish susceptibility to anthracnose in banana (Chillet *et al*., 2000). The other reports indicated that postharvest used of calcium diminish anthracnoses disease in Papaya (Mahmud et al., 2008). Boron is essential for plants that it plays several consequential roles in plants metabolic changes and cell damage in the affected tissue (Marschner, 1986). It also participates in structure of cell wall formation and division, carbohydrates transport, improvement of plant sexual growth, development of hormone system and control of phenol metabolism (Ahmad, 2009).

Although numerous physiological disorders reported by pistachio growers in all over the world but little evidence was accessible in the reply of the pistachio tree to the application of calcium nitrate, calcium chloride and boric acid in order to control nut physiological disorders. Therefore, the objective of this study was to distinguish the effect of calcium nitrate, calcium chloride and boric acid and moreover their combinations on physiological disorders of pistachio fruit.

## Materials and Methods

### *Plant material and experiments*

The experiments were done on 13-year-old 'Kaleh-Ghoochi' pistachio trees grafted on Badami-Riz (Pistacia vera cv Badami-Riz) rootstock at the Sharif abad orchard located in Qom, Iran. In 2017, thirty trees were chosen and six treatments were applied on the trees, including (1) control, (2) calcium nitrate; 2 gr/lit, (3) calcium chloride; 2 gr/lit, (4) boric acid; 200 ppm, (5) combination of calcium nitrate; 2 gr/lit and boric acid; 200 ppm, (6) combination of calcium chloride; 2 gr/lit and boric acid; 200 ppm .Foliar application were done one week earlier full bloom and also two weeks later than full bloom. All horticultural practices were followed during the experiment. On each tree, five quite homogeneous shoots were selected and labeled. Each chosen shoot contains three clusters. The results of soil analysis of orchard were shown in Table 1.

**Table 1. The soil characteristics of the experimental orchard.**

| Soil texture | depth (cm) | soil pH | soil EC (ds. $m^{-1}$) | K mg/kg | Na mg/kg | Mg mg/kg | Ca mg/kg |
|---|---|---|---|---|---|---|---|
| silty- clay | 0-30 | 7.8 | 9.63 | 58 | 228 | 272 | 1009 |

### *Estimation of disordered nuts*

The percent of fruit set and the percent of fallen fruits on each fruit cluster were estimated two weeks later than fruit set and at harvest time, respectively. Fruit abscission (%) was estimated through dividing the number of fallen fruits by the primitive number of fruit set on each shoot. Other pistachio disorders only were estimated at harvest time on each clusters. Since the early-split disorder happened nearly four weeks earlier harvest time, the number of early-split nuts was gauged earlier to harvest. When the nut-shells was easily detached from the endocarp, pistachio nuts were harvested (Ferguson *et al*., 2005), all the fruit clusters were detached from shoots and hand sorted in to blank, non-split, deformed, endocarp lesion and split nuts. To sort blank and non-split nuts, they were floated in ethanol. The disorders were reported as the percentage of disordered fruits by all the fruit sets.

### *Biochemical traits*

To assay biochemical traits, sampling the fruit was taken on the first of August. According to the method of Obinger *et al.* (1997), Catalase activity was assayed according to the method described by Chance and Maehly (1955), Poly phenol oxidase activity was assayed. According to the method described by Kar and Mishra (1976), peroxidase activity was assayed with some modifications. According to the method described by Saunders and Mcclure (1974), the activity of phenylalanine ammonilyase enzyme was

assayed. In this experiment, the total phenol ingredients was assayed by Folin Ciocalteu's method (Singleton and Rossi, 1965), and also Proline ingredients was assayed according to the method described to Lattanzio *et al*. (2009).

***Statistical analysis***

In this experiment, to find the effects of treatments on each dependent variable, randomized complete block design (RCBD) was used with 5 replications. Analysis of variance was done by using SAS software (version 9.2) and mean values of treatments were compared using the Least Significant Difference test (LSD, P=0.05).

**Results**

***Effect of treatments on nut disorders***

In this experiment results specified that all applied treatments as compared to control diminished the fruit abscission. The effect of calcium nitrate was more than effects of calcium chloride in reducing the fruit abscission. While there were no considerable differences between calcium nitrate and boric acid in fruit abscission. On the other hand, there were no considerable differences between calcium nitrate and boric acid and combinations treatments in the fruit abscission (Fig. 1).

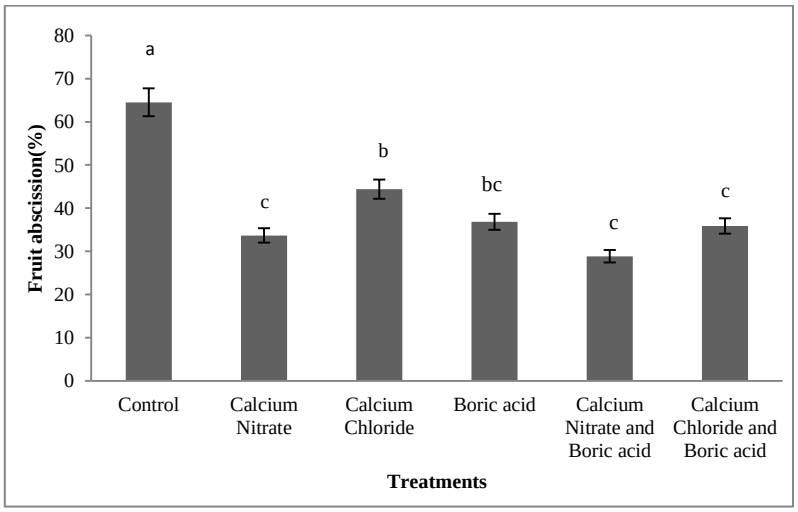


**Fig. 1.** Effect of foliar application of Calcium Nitrate, Calcium Chloride and Boric Acid on fruit abscission

According to the experiment, all applied treatments, excepting boric acid as compared to control, diminished endocarp lesion in pistachio nuts but there were no considerable differences among treatments in decreasing endocarp lesion in pistachio nuts (Fig. 2).

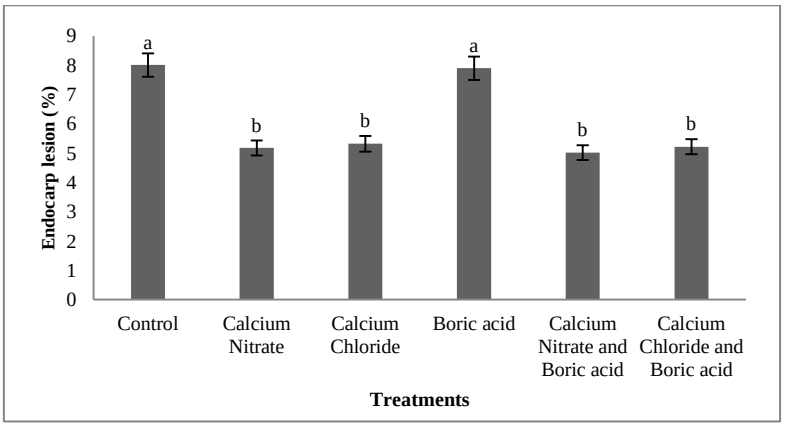


**Fig. 2.** Effect of foliar application of Calcium Nitrate, Calcium Chloride and Boric Acid on endocarp lesion.

In the case of blank nut, results specified that all treatments diminished the blank nuts as compared to control, but no considerable differences were observed between the all treatments (Fig. 3).

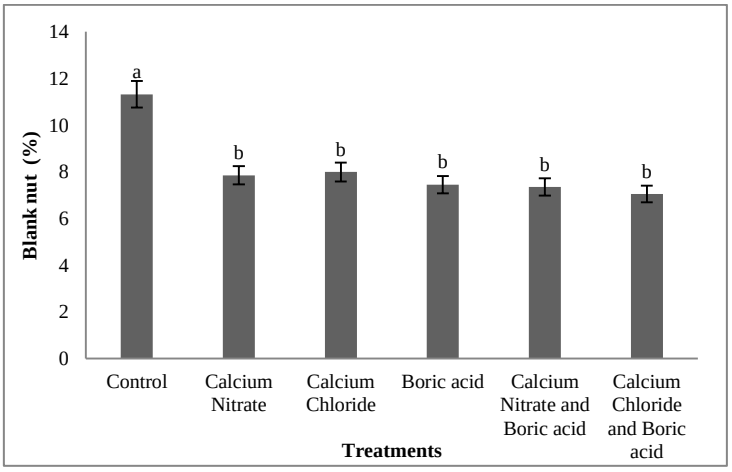


**Fig. 3.** Effect of foliar application of Calcium Nitrate, Calcium Chloride and Boric Acid on blank nut.

Generally, low level of deformed nuts was observed in this study. However, the results specified that calcium nitrate and moreover combination treatment of calcium nitrate and boric acid diminished the number of deformed nuts as compared to other treatments and control. So, there was not a considerable difference between other treatment and control in the number of deformed nuts (Fig. 4).

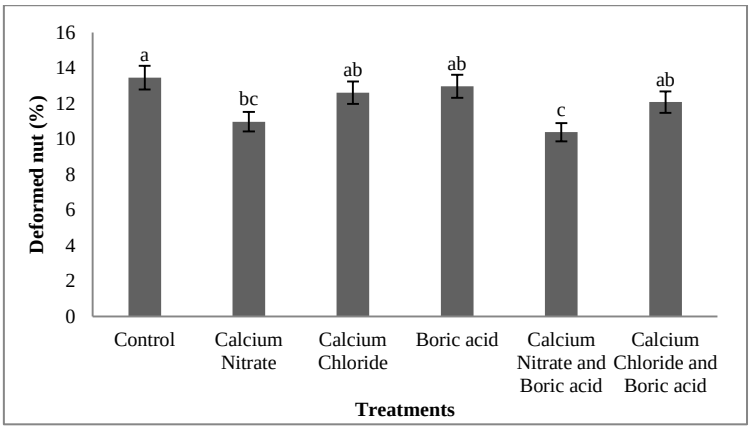


**Fig. 4.** Effect of foliar application of Calcium Nitrate, Calcium Chloride and Boric Acid on deformed nut.

In the case of early split nut, the results specified that all applied treatments except to boric acid, diminished this disorder in comparison to control. but no considerable differences were observed between the all treatments (Fig. 5).

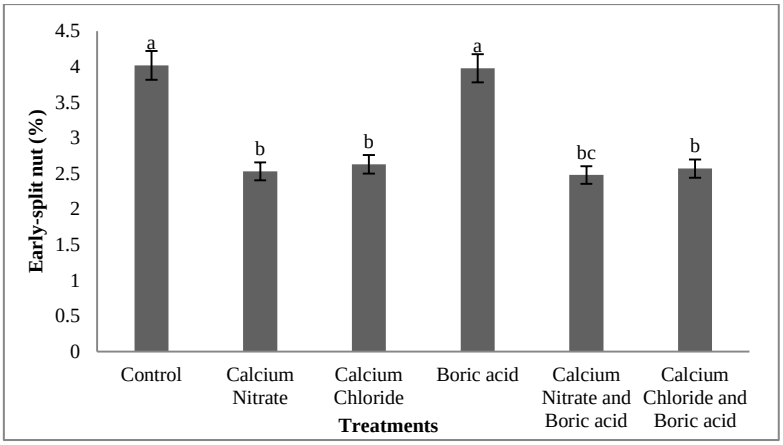


**Fig. 5.** Effect of foliar application of Calcium Nitrate, Calcium Chloride and Boric Acid on early split nut.

Also according to the experiment, excepting boric acid treatment, all applied treatments, diminished non-split nut in comparison to control. On the other hand, no considerable differences were observed among the other treatments (Fig. 6).

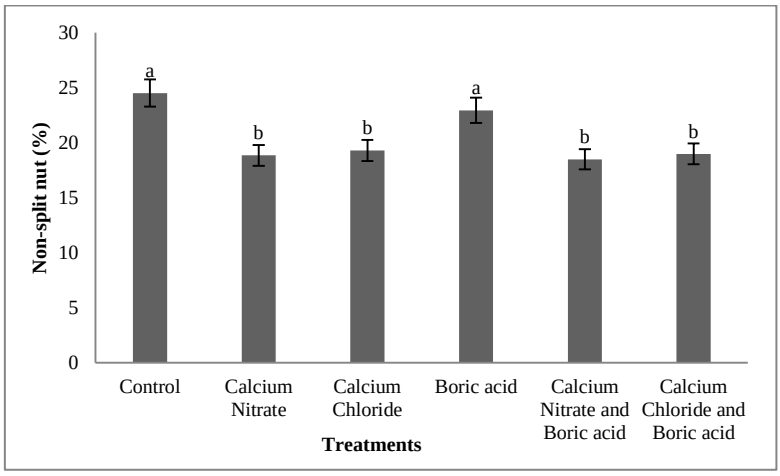


**Fig. 6.** Effect of foliar application of Calcium Nitrate, Calcium Chloride and Boric Acid on non-split nut.

In the case of split nut, the results specified that excepting boric acid treatments, all applied treatments in comparison to control, boosted split nuts. But there were no considerable differences between calcium nitrate and calcium chloride to boost the split nuts but the best result was obtained through the application of calcium nitrate and boric acid (Fig. 7).

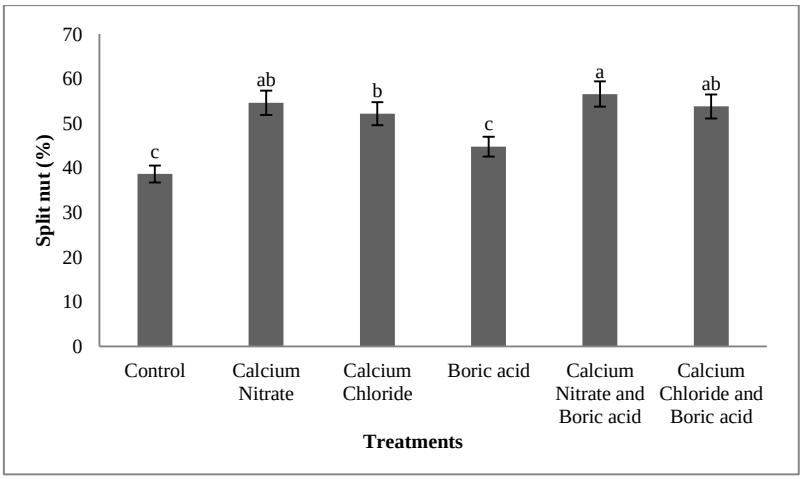


**Fig. 7.** Effect of foliar application of Calcium Nitrate, Calcium Chloride and Boric Acid on split nut.

***Effect of treatments on biochemical traits***

In this study, the results specified that all the treatments diminished the CAT enzyme activity as compared to control. The effect of calcium nitrate was much more than calcium chloride and boric acid to diminish CAT activity, and also the effect of calcium chloride was more than the boric acid to diminish CAT activity. Among the combination treatments, calcium nitrate and boric acid had more than the effect of calcium chloride and boric acid to diminished CAT activity (Table 2).

**Table 2. Effects of foliar application of Calcium Nitrate, Calcium Chloride and Boric Acid on biochemical factors of 'Kaleh-Ghoochi' pistachio.**

| Treatments | CAT (u/mgFW) | POD (µg.min/gFW) | PPO (µg.min/gFW) | PAL (nmol.min/gFW) | Total phenol(mg/gFW) | Proline (µg/gFW) |
|---|---|---|---|---|---|---|
| Control | 8.32$^{a}$ | 6.28$^{a}$ | 4.96$^{a}$ | 10.19$^{a}$ | 1.34$^{a}$ | 11.94$^{a}$ |
| Calcium nitrate | 6.75$^{d}$ | 4.59$^{c}$ | 4.17$^{c}$ | 8.95$^{c}$ | 0.95$^{d}$ | 8.23$^{c}$ |
| Calcium chloride | 7.52$^{c}$ | 5.38$^{b}$ | 4.41$^{bc}$ | 9.27$^{bc}$ | 1.17$^{b}$ | 9.78$^{b}$ |
| Boric acid | 7.92$^{b}$ | 6.09$^{a}$ | 4.55$^{b}$ | 9.87$^{b}$ | 1.23$^{b}$ | 11.75$^{a}$ |
| Calcium nitrate and Boric acid | 6.23$^{e}$ | 4.09$^{cd}$ | 3.71$^{d}$ | 7.87$^{d}$ | 0.88$^{d}$ | 8.12$^{c}$ |
| Calcium chloride and Boric acid | 7.32$^{cd}$ | 5.19$^{b}$ | 4.19$^{c}$ | 5.95$^{e}$ | 1.06$^{c}$ | 9.58$^{bc}$ |

In each column, means with the same letters are not significant according to Duncan's multiple range test ($P<0.05$).

The results of POD enzyme activity specified that except to boric acid, all applied treatments, diminished POD activity as compared to control. The effect of calcium nitrate was much more than calcium chloride in diminishing of POD activity. On the other hand, combination treatment of calcium nitrate and boric acid was more than the effect of other solitary and combinations treatments to diminish POD activity (Table 2).

According to the experiment, PPO activity was diminished under the effects of application treatments as compared to control. Also, the effect of calcium nitrate was more than calcium chloride and boric acid, and also the effect of calcium chloride was more than boric acid to reduce of PPO activity. However, combination treatment of calcium nitrate and boric acid was more than effect of other solitary and combinations treatments to diminish PPO activity (Table 2).

In this study, samples had the lower levels of PAL activity than control samples, the effect of calcium nitrate was more than effects of calcium chloride and boric acid and also the effect of calcium chloride was more than boric acid in diminishing of PAL activity. In combinations treatments, the lowest PAL activity was observed during the treatment of calcium nitrate and boric acid (Table 2).

According to the experiment, treatments diminished total phenol ingredients as compared to control. However, the effect of calcium nitrate was more than calcium chloride and boric acid to diminish total phenol content. Also, no considerable difference was observed between calcium chloride and boric acid to diminish total phenol ingredients. On the other hand, among combination treatments, calcium nitrate and boric acid diminished total phenol ingredient which was considered more effective than calcium chloride and boric acid treatments (Table 2).

All samples had lower proline ingredient as compared to control samples. The effect of calcium nitrate was more than calcium chloride and boric acid and also the effect of calcium chloride was more than boric acid to diminish the proline ingredients. Among combination treatments, calcium nitrate and boric acid diminished the proline ingredient which was more effective than calcium chloride and boric acid treatments. No considerable difference was observed between combination treatments, calcium nitrate and boric acid and calcium nitrate in the proline ingredient (Table 2).

**Discussion**

In this study, the results of soil analysis of orchard have specified that the grade of water and soil EC are high than over limit. Pistachio is commonly famous as tolerant trees to salinity and drying but the high salinity and drying diminishes product and boost disorders in pistachio orchards (Herrera, 1997). Our results have specified that there is a junction among physiological disorders in pistachio nuts with PAL, PPO, POD, CAT, proline and phenol ingredient of nut-shells and leaves. It seems that among physiological disorders in pistachio and surrounding stresses, there is a junction.

Plants in unfavorable situations such as biotic and abiotic stresses produced Reactive Oxygen Species (ROS) which induce damage to cellular structures. Versus environmental stresses, plants used enzymatic and non-enzymatic mechanisms (Pazuki *et al*., 2015). In fact PAL, PPO, POD and CAT are antioxidant enzymes that created in stress conditions. In stress condition, antioxidant enzymes prevent from more damages to cellular structures through eliminating such dynamic oxygen species as $H_2O_2$ and counterbalance deleterious free radicals. On the other hand, PAL has been presented as the key enzyme of phenylpropanoid traverse causing to the production of antioxidant metabolites like ascorbic acid, phenolic complexes and flavonoids (Queiroz et al., 2008; Amna Mhamdi et al., 2010; Kim and Hwang, 2014; Siddika *et al*, 2015). Phenolic complexes are non-enzymatic mechanisms which have a consequential role in the interplay of plants with its surroundings. In fact these complexes defended plants versus biotic and abiotic stresses (Kliebenstein, 2004). Proline is a critical material that diminished disorders through expanding the scope of stresses in agriculture in situation stresses (Pazuki *et al*., 2015). In this study, the results have specified that treatments diminish physiological disorders and biochemical indexes.

Our results have specified that all applied treatments diminished the percentage of fruit abscission. Some reports specifies that boron has an significant role in the proceeding of fertilization and it has been reported that boron shortage in style and stigma diminishes pollen germination and growth of the pollen tube in process of fertilization in which it causes the fruit abscission (Nyomora and brown, 1997). In fact boron prevents the action of indole acetic acid oxidase, causes the persistency of auxins in plants tissue, and diminishes fruit abscission (Castro and Sotomayor, 1997). Another study has indicated that number of the fruit, usually are boosted in pistachio trees with the advancement of the nitrogen supply, (Arias *et al*., 2005). Jackson (2003) reported that consumption of urea can diminish the fruit abscission in apple and pear trees. In fact the presence of nitrogen has an important role in the process of fertilization and fruit set.

Calcium has been important to improve fertilization, and subsequent embryo and fruit improvements (Anil and Rao, 2001). Pollen often needs on calcium sources in the pistil for germination and elongation of the pollen tube. On the other hand, free calcium in cytosol is an important secondary messenger in cell signaling. In fact it systemizes the polarity of the pollen tube and abundance of calcium signals in ovary and ovule, provides essential mineral nutrition, and shifts the pollen tube in some plants (Lili *et al*., 2007; Fageria, 2009; Dodd *et al*., 2010). On the other hand, the unfavorable environmental situations, minerals and growth adjustments have been reported as some rationales for the fruit abscission (Acar and Eti, 2007). In environmental stresses, plants produce ROS and ethylene which acts as an intracellular signal to stimulate plant protection mechanisms versus kind 0f stresses (Chen *et al*., 2005; Xia *et al*., 2015). It seems that the boost of the fruit abscission has a positive correlation with ethylene. Since calcium acts as anti-ethylene in abscission process (Taiz, and Zeiger, 2015), In fact calcium in treatments diminishes the rate of stresses, so the fruit abscission was diminished.

In this research, the percentage of the endocarp lesion disorder is diminished with application of all treatments excepting boric acid. With studying different sources of calcium such as calcium chloride and calcium nitrate on endocarp lesion disorder. Sajadian and Hokmabadi (2011) reported that these treatments diminished the rate of disorder as the study has been shown. Gastol and Iwona (2006) who studied the rate of calcium in the different parts of the pear fruit reported that the highest amount of calcium was the presence on the skin of fruit. Also, these studies have shown that the presence of calcium was essential when cell membranes were damaged through various stresses (Netonda *et al*., 2004). Usually the soak up of calcium into nuts is done with mass flow process and is related to transpiration. In salinity and drought situations, the soak up of water and calcium is limited. In fact spray of calcium nitrate and calcium chloride are boosted in pistachio fruits and the endocarp lesion disorder is diminished. However, the causes of the endocarp lesion disorder in pistachio are not plainly known.

Our study has been showed that all treatments diminish the percentage of the blank nuts. Some of the researchers reported that fertilization challenge such as destruction of the ovary segments, are the chief factor of blanking in pistachio (Shuraki and Sedgley, 1996). Some of the researchers reported that when the movement of pollen tube is stopped in the style, the particle of starch is promptly aggregated in an ovary, then blanking happen in pistachio (Shuraki, 2006). Boron deficiency limits the reproductive growth, boosts the weak anther, develops the defective pollen, and stops the growth of the pollen tub (Ahmad *et al*., 2009). Moreover, some researchers reported that blanking is created at the time of fruit set and to complete the kernel (Ferguson *et al*., 2005). It seems that boron diminishes percentage of the blank nuts through improving the fertilization and the nutritional situations if blank nut is association to watering and nutritional stresses (Ferguson *et al*., 2005).

Calcium was prevented from some of physiological processes of plant (Hashimoto and Kudla, 2011). Some researchers reported that biotic and abiotic stresses boosted intracellular calcium source and induce cell cycle delay (Sano *et al*., 2006). Also, calcium could reinforce drought tolerance and diminishing the proline oxidase activity (Abdul Jaleel *et al*, 2007). On the other hand, nitrogen is the main source of amino acids and proline is one of amino acids which in response to drought and salinity stresses were accumulated in plant tissue and boosted plant resistance to stresses (Verslues *et al*., 2006). However, few researches have reported the precise causes of blanking in pistachio nuts.

Some report specifies that the deformed nuts in pistachio are generated with disease, insect or other physical damages (Niven *et al*., 1994). Microscopic perceptions showed that deforming in pistachio has been established two weeks later than full bloom with destruction of parenchymal cells in endocarp (Metheney *et al*, 1995). Calcium as a nutrient has chief role in cell functions, in association, Fageria (2009) reported that calcium was essential in cell division and elongation, the structure of the cell wall in plants and other organelles and it also helped the activity of auxin. On the other hand, auxin is significant to improve the shape of the fruit (Tiwari *et al*., 2012). According to the results of experiment, It seems that calcium diminish percentage of the deformed nuts through participating in rebuilding of the tissue. However, little research has been reported about the precise causes of deformation in the pistachio nut.

Some report specifies that the causes of boosting early-split are the nutrition and irrigation stresses in an orchard (Doster *et al*., 2001; Hosseinifard and Panahi, 2006). Our results specified that treatments, especially calcium nitrate + boric acid and calcium nitrate, diminish the percentage of early split nut. If the early split nut is associated to the nutrition and irrigation stresses, it seems that our treatments can possibly diminish the nutrition and irrigation problem. In stress situations, the rate of ROSs and ethylene is boosted (Perez-Lopez *et al*., 2009; Xia *et al*., 2015). ROSs hurts membranes, lipids, proteins, nucleic acids,

cellular and intracellular structures (Perez-Lopez *et al*., 2009; Karray-Bouraoui *et al*., 2011). Since in early-split nut affects the skin of the fruit, it seems that ROSs and ethylene motivate the rate of early-split nut to control the pistachio trees.

## Conclusions

It is important to control the physiological problems in pistachios. The results of the experiment show that environmental stresses are effective on physiological problems. Also, the results specify that treatments, especially combination treatment of calcium nitrate and boric acid, diminish the physiological problems through reducing stress situations, and the synergistic effect of calcium nitrate + boric acid is better than each one alone. The other treatments have fewer effects on physiological problems.

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